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THERMOMECHANICAL ANALYSIS OF STRATUM CORNEUM

I. TECHNIQUE*

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ABSTRACT

The technique of thermomechanical analysis has been applied to the study of neonatal rat stratum corneum from 25° to 325° C. The membrane has been examined in the penetration, extension, and expansion modes.

In the temperature range studied, the material emphasized its anisotropic structure by exhibiting a softening at 45° C and 155° C, a 404% expansion at 213° C in transverse measurements and a 1% contraction at 50° C and 196° C followed by extension at 222° C in the longitudinal direction with a complete loss of modulus at 285° C.

The coefficient of linear expansion is reported to be relatively constant over a 126° C range.

The thermally induced viscoelastic as well as dimensional transitions further characterize the stratum corneum and should aid in elucidating the molecular mechanisms responsible for its biomechanical properties.

The formation of the stratum corneum by the keratinization process is characterized by the production of a highly stabilized, mechanically strong and chemically resistant biological membrane. Determination of the relative contributions the macromolecular components make to the observed macroscopic properties of stratum corneum are under investigation (1). The physical and chemical transformations induced in stratum corenum and proteins on heating from ambient temperature (20° C) to 250° C have been studied by the use of several thermoanalytical techniques (2, 3). Differential thermal analysis (DTA) of stratum corneum was shown by Bulgin (4) to have several endotherms which were apparently due to phase transitions within the membrane mosaic. Thermogravimetric analysis (TGA) (5) of stratum corneum indicates that in the temperature range from ambient to approximately 200° C, the loss of volatile components other than water are negligible. Volatile decomposition products are formed beginning at about 200° C.

Middleton (6) measured the influence of equilibrium temperature on the flexibility of hydrated guinea pig stratum corneum from the footpad. There was a significant decrease in the flexibility when the temperature was reduced below ambient. Baden (7) and Rudall (8) measured the thermally induced isometric contraction of highly hydrated stratum corneum at about 90° C. X-ray diffraction (7) suggests that this phenomenon is due to conversion of the macromolecular structure of the alpha-keratin to the beta configuration. This observation is similar to the "shrink temperature" (9) which is observed in collagen containing tissues, where the alpha helical collagen is transformed to a beta configuration. All of these measurements take advantage of the macromolecular character of stratum corneum as influenced by temperature.

Thermomechanical analysis (TMA) of the intact stratum corneum membrane complements other thermoanalytical techniques mentioned. TMA monitors temperature dependent properties such as dimensional and viscoelastic changes which may not be sensed by gravimetric (TGA) or enthalpic (DTA, DSC) thermal analyzers and allow the calculation of such fundamental properties as the coefficient of linear expansion.

The purpose of this work is to demonstrate the applicability of this technique in elucidating the structure and forces involved in stratum corneum stability. The influence of sorbed water on these properties is minimized in this study by using samples conditioned at ambient relative humidity which contain about 10% water by weight.

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MATERIALS AND METHODS

Stratum corneum preparation. The newborn rat stratum corneum used in these studies was isolated from 24 to 36 hour old rats according to the procedure used by Vinson (10). The separation procedure involves exposure of the isolated full thickness skin to ammonia vapors for 30 minutes after which the dermis separates easily from the epidermis. The adhering wet cells were gently removed with a cotton swab and the stratum corneum sheet allowed to dry at ambient conditions on a sheet of silicone-coated paper.

The isolated sheets of stratum corneum were equilibrated at ambient conditions (RH = 30–40%, T = 23–25° C) for at least 48 hours prior to their use. Samples of appropriate sizes for analysis were prepared by cutting discs of 6mm diameter for both the expansion and penetration studies and 2 x 9 mm strips for the extension runs.

Measurements. The thermomechanical analysis determinations were conducted with a Perkin Elmer Model TMS-1 Thermomechanical Analyzer equipped with a Texas Instrument "Sevro-Riter II" two-channel Potentiometer Recorder. A schematic of the equipment is shown in Figure 1. Basically, the technique involves the use of a weighted probe resting on the sample and measurement of its linear displacement as the material is heated at a programmed rate. The maximum sensitivity of the system is such that a probe displacement of 1.2 μ will cause a full scale deflection (10 inches) of the pen.

The disc of stratum corneum was placed into an aluminum pan and positioned on the sample platform of the TMA. A weighted probe was lowered onto the material and the furnace raised into position surrounding the sample. The probe was then tapped lightly to insure proper seating on the surface of the sample. The TMA sample compartment was constantly flushed with dry helium at a rate of 50 cc/min. to prevent condensation and reduce the thermal gradient between the furnace and the sample. The system was then heated at a constant rate and the linear displacement of the probe continuously recorded graphically as a function of temperature. The temperature of the sample was monitored by a chromel-alumel thermocouple positioned approximately 3 mm above the sample.

The three modes of operation, expansion, penetration, and extension utilized separate probes specially designed for optimum measurement of specific viscoelastic and dimensional properties. The configuration of the three probes are shown in Figure 2. The penetration probe has a tapered end with a round tip (1 mm diameter) to allow for application of the stress onto a small area of the sample surface. The expansion probe has a flat end (4 mm diameter) to rest on the sample surface and reflect any change in thickness dimensions. The extension mode has the sample strip (8 mm in length) suspended between a fixed and a movable probe. Changes in sample length are recorded by displacement of the movable probe.

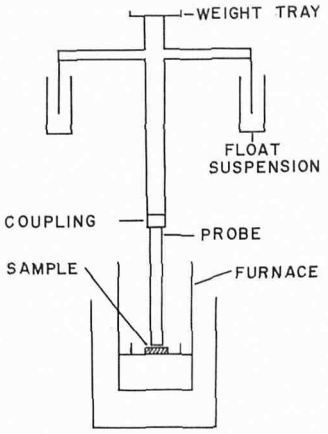

FIG. 1. Schematic of thermomechanical analyzer.

RESULTS

Calibration. The thermal mechanical system was calibrated by determining the coefficient of linear expansion for an aluminum standard. The experiment incorporated the expansion probe with a load of three grams. The three gram load overcomes the buoyancy force that mechanically supports the probe.

The TMA thermogram for a calibration run is shown in Figure 3. From this experiment, the coefficient of linear expansion was calculated to be 24.7 $\times$ 10^{-6} inches/inch° C. The literature value is reported to be 24.2 $\times$ 10^{-6} inches/inch° C.

Operating conditions. A series of initial experiments was conducted to determine the optimum operating conditions. The effect of heating rate and load on the temperature at which the penetration probe finally penetrated through the sample is shown in Figures 4 and 5. Acceptable sensitivity was obtained at 20° C/min. and a load of three grams. Sharp thermal transitions were produced with this combination of load and heating rate.

The thermograms obtained in this type of a study will be dependent upon the applied stress. If the applied stress is greater than the samples' modulus then thermal transitions may become obscured.

Penetration study. An actual thermogram for a typical penetration run on rat stratum corneum is shown in Figure 6. There is an initial penetration at 45° C, another softening begins at 155° C, an expansion above 200° C followed by final penetration at 285° C. Complete penetra-

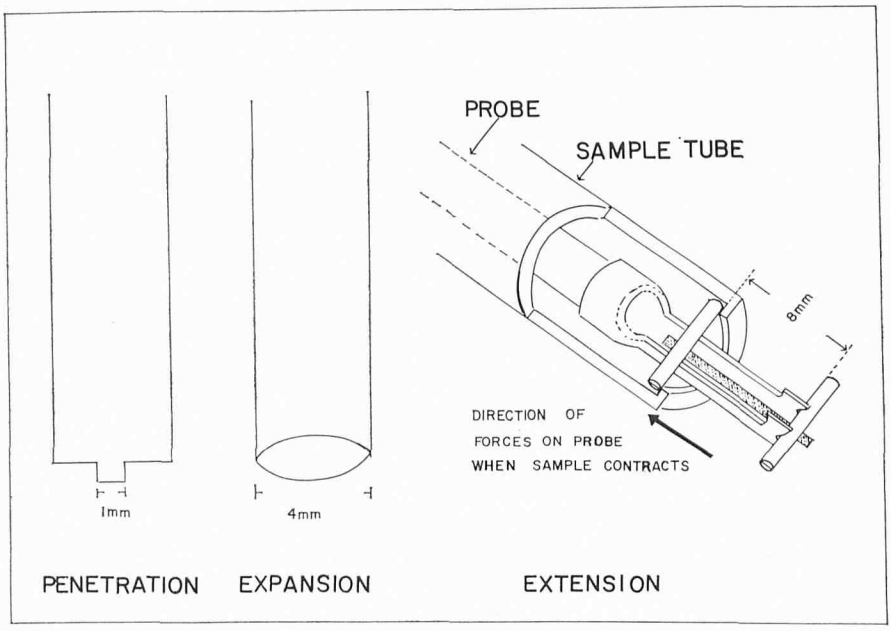

FIG. 2. Thermomechanical analysis probe configurations

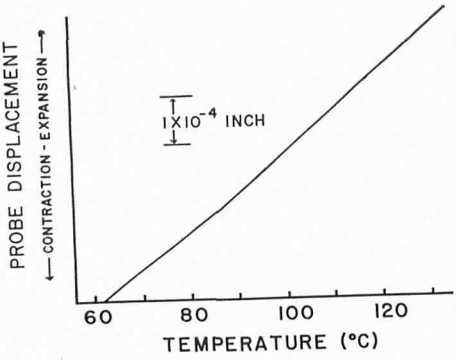

FIG. 3. Calibration of TMA with aluminum standard. Operating conditions: 10° C/min., 3 gm load sample height = 0.298 inch.

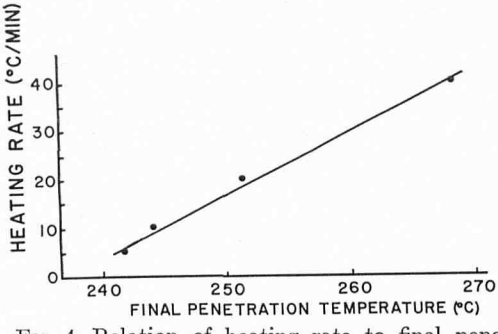

FIG. 4. Relation of heating rate to final penetration temperature for rat stratum corneum at a constant load of 5 grams.

tion of the sample by the probe was verified by observation of the sample in the pan with a binocular stereoscope following the run. The total displacement of the probe for the penetration process gives an average sample thickness value.

The expansion observed in the penetration mode does not clearly reflect the transition since the membrane also softens at that temperature allowing the probe to penetrate and therefore mask the phenomena. The expansion probe with a layer of silica over the sample to prevent localized penetration allows a more detailed study of the transition.

The thermally induced phase transitions observed between ambient and 213°C were examined for reversibility. The experiment involved preheating the samples to temperatures just beyond the initiation of each transition, 47, 157, and 227°C, holding the sample at that temperature for two minutes, cooling and then running a penetration measurement on each sample. Preheating the sample to 47°C, just beyond the first transition, resulted in no significant change in the penetration thermogram. The sample preheated to 157°C had a normal thermogram with the exception of a very much reduced initial penetration at 45°C. The thermogram for the 227°C preheated sample showed a penetration at

155°C without the presence of the 45°C penetration. The reversibility of the 155°C transition may indicate that the observed phenomena may approximate a true crystalline melt and that the melted material is stable to at least 227°C.

Expansion study. The expansion at 213°C indicated by the penetration probe was further examined with the expansion probe. The expansion thermogram is shown in Figure 7. The expansion was found to be 404% of the original sample thickness with a standard deviation of 16%.

The penetration and expansion data were examined statistically to determine the reproducibility of the samples and measurement system. The standard deviation for each inflection temperature as well as the linear displacement of the probe at the end of the transition are shown in the Table. The linear displacement of the probe was normalized to the actual sample thickness as determined from total displacement for complete penetration. Sample thicknesses varied from 9 to 12 microns. The low standard deviations reflect the precision of the system as well as the consistency of the stratum corneum membranes.

Extension study. An extension thermogram is shown in Figure 8. The sample exhibited an initial contraction (1%) beginning at 50°C with a second contraction at 196°C followed by rapid extension at 222°C.

Coefficient of linear expansion. The coefficient of linear expansion (α) of stratum corneum was determined for the transverse direction relative to the flattened cells. The coefficient of linear expansion used here is the ratio of the change in thickness per degree C to the thickness at 20°C. The reference thickness at 20°C was chosen rather than the conventional 0°C as a matter of convenience and to eliminate any complicating irreversible effects due to cooling the sample to 0°C. Loads which were slightly greater than the internal buoyancy of the system were used in order that the probe would seat on the sample and follow the dimensional changes as the sample was heated. The material was placed in the furnace and cooled to 8°C and maintained at that temperature for 30 minutes prior to heating at 20°C/min.

The coefficient was found to be -3.18×10^{-3}°C^{-1} over the range from 39 to 176°C except for the region of 70 to 86°C where a significant modulus change occurs in the sample.

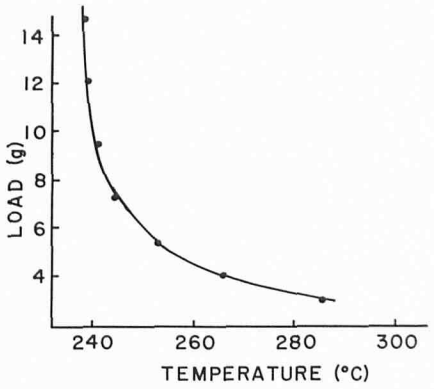

FIG. 5. Relation of load to the final penetration temperature for rat stratum corneum at a constant heating rate of 20° C/min.

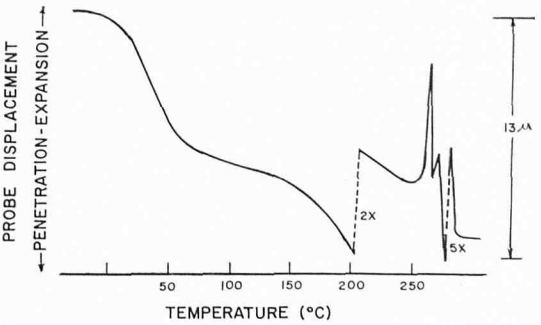

FIG. 6. Penetration thermogram for rat stratum corneum. Operating conditions: 20° C/min., 3 gm load.

DISCUSSION

The temperature at which certain physical properties of solids such as bulk modulus, dimensions and viscoelasticity show abrupt changes has long been recognized as an indicator of the component stabilizing forces. This temperature is commonly referred to as a transition temperature. Hence, melting temperature of crystalline materials is a measure of the kinetic energy required for disruption of the stabilizing intermolecular attractive forces and is characteristic of the particular solid material. Inter and intra molecular forces of long chain polymers stabilizing a three-dimensional network such as the fibrous proteins, mucopolysaccharides and lipids which are structural components of stratum corneum are dependent on both the specific attractive forces and the geometry of the long chain polymers. It is the thermodynamic considerations of the latter aspect (spatial configuration) which is most critical in determining these

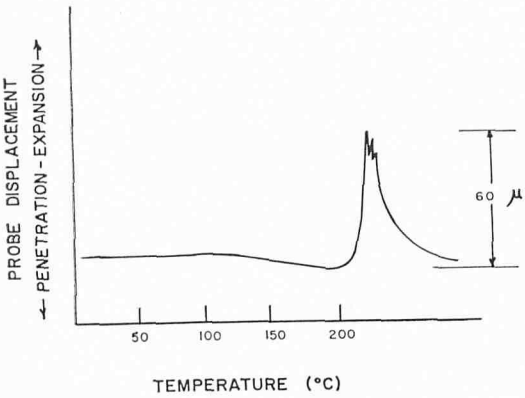

FIG. 7. Expansion thermogram for rat stratum corneum. Operating conditions: 20° C/min., 3 gm load.

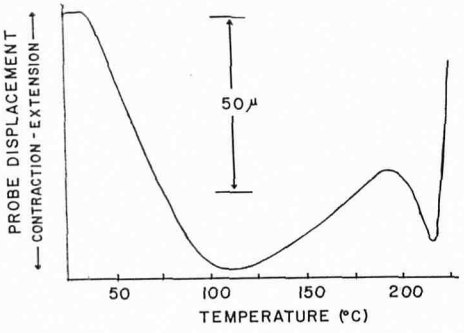

FIG. 8. Extension thermogram for rat stratum corneum. Operating conditions: 20° C/min., 2.3 gm load, sample length = 0.8 cm.

TABLE

Relationship between inflection temperature, standard deviation and the percent displacement of the TMA probe

Transition	Inflection temperature (°C)	Probe displacement (%)
Penetration	45 ± 3	17.8 ± 3.8
Penetration	155 ± 12	51.5 ± 7.2
Expansion	213 ± 15	404 ± 16
Penetration	285 ± 5	100 ± 5.3

thermally induced transitions in polymeric systems.

Softening of polymeric materials is due to the introduction of enough kinetic energy into the material to cause increased segmental motion of the molecular chains decreasing their interactions with adjacent chains and resulting in a lower modulus to shear and tensile deformation. Operationally this is observed by the TMA when upon heating the sample, the weighted penetration probe causes sample deformation indicating a thermally induced modulus change.

Since the stratum corneum is highly anisotropic in structure due to the preferential orientation of the keratin, the influence of temperature on the viscoelastic properties might be expected to vary depending on whether the measurements are made through the thickness of the sample, as with the expansion and penetration modes, or in the longitudinal plane of the cell, as with the extension mode. As can be seen from the data, the initial softening which occurs at 45°C is accompanied by a 1% contraction longitudinally with no abrupt change in sample thickness. At 196°C the sample contracts for a second time and at 222°C it begins to elongate with increasing temperature. Finally, at 285°C the modulus becomes so low that the penetration probe penetrates the sample completely. The softening which occurs at 155°C is not accompanied by any other observable changes in viscoelastic properties measured.

From studies of the similarities observed in the thermal behavior of simple proteins and wool keratin, it has been suggested that a phase change which represents an irreversible alpha-helix disordering transition in simple proteins occurs in the region of 200 to 250°C in wool (12, 13). Therefore, it is tempting to speculate that the expansion and softening which occurs at about 215°C in stratum corneum also represents a disordering of the highly organized alpha-keratin. However, further evidence is necessary to substantiate this explanation.

Viscoelastic changes which occur in highly hydrated samples of stratum corneum such as observed by Baden (7) and Rudall (8) will not necessarily occur at the same temperature in the dry state. In dry samples, it is reasonable to expect that the energy of activation for segmental diffusion would be high and the rate of diffusion lower. The water solvates the polar groups in the polymeric chains, as well as mechanically separates charges through swelling, resulting in a reduction in the attractive forces. The effect would be to reduce the energy barrier necessary for the transition; hence, it occurs at a lower temperature when hydrated.

The reproducibility of the distinct transitions in stratum corneum with all three modes of

TMA operation suggests a high degree of macromolecular organization within the membrane mosaic. This technique applied to diseased and chemically altered stratum corneum should further contribute to the understanding of the macromolecular organizational defects present in some dermatoses.

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